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Original Research

PHYTOCHEMICAL SCREENING, PHENOLICS AND FLAVONOIDS QUANTIFICATION, AND FREE RADICAL SCAVENGING POTENTIAL OF *EMILIA PRAETERMISSA* MILNE-REDHEAD (ASTERACEAE) LEAF

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ABSTRACT

Introduction: Phytochemicals have been linked to the activities exhibited by extracts and fractions from plant in *in-vivo* and *in-vitro* experiments. Phenols and flavonoids, two important phytochemicals are implicated in the treatment of different illnesses. They contain at least an aromatic ring and hydroxyl group in their molecules, which are relevant moiety in their *in-vitro* quantification. *Emilia praetermissa,* an annual herb, use in traditional medicine for the treatment of varieties of ailments which are oxidative stress related.

Aims: This study aims to evaluate qualitatively phytochemicals in the extract and fractions, limit the quantitative determination of the phytochemicals to total flavonoids and phenolic contents, and evaluate their 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.

Methods: The methanol extract and fractions obtained from liquid-liquid partitioning of the extract with n-hexane, dichloromethane and ethylacetate were screened for phytochemicals by using preliminary established screening methods. While the level of flavonoids and phenolic compounds were determined spectroscopically using 10 % aluminum chloride and 10 % Folin-Ciocalteu reagents. Antioxidant potential was evaluated by the ability of the extract and fractions to scavenge for free radicals from DPPH.

Results: Flavonoids and phenolic compounds were identified in the extract and fractions, and quantitatively dichloromethane fraction showed the highest level for phenols 60.94±0.22 mg GAE/g and flavonoids 19.43±0.06 mg QE/g. Radical scavenging potential of the n-hexane fraction compared favorably with the standard, methanol extract and dichloromethane fraction ($p < 0.05$), and IC50 of 30.00 µg/mL was recorded for dichloromethane fraction.

Conclusion: This study shows that *Emilia praetermissa* leaf contains varying classes of phytochemicals especially flavonoids and phenols that can scavenge for free radicals.

Keywords: *Emilia praetermissa*, Antioxidant, Total Phenolic Content, Total Flavonoid Content.

INTRODUCTION

Free radicals in high concentration result in compromise of the cellular structure; this can bring about diseases such as cancer, rheumatoid arthritis, congestive heart failure, cardiomyopathy, hypertension and ulcer. Antioxidants mitigate the actions of free radicals, thus plummeting or revising the consequence of their production [1,2,3,4,5,6]. Meaning that plants rich in antioxidant, could be used in the managing or treatment of some of these diseases. By nature antioxidants are phytochemicals that scavenge for free radicals such as phenolics and flavonoids compounds. These compounds have been use in treating or managing oxidative stress

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related diseases [7]. Many plants are rich sources of flavonoids and phenolic compounds with at least one hydroxyl group attached to an aromatic ring [8].

Emilia praetermissa Milne Redhead (Asteraceae) is an herbaceous plant commonly called "yellow thistle flower", in western Nigeria it is referred to as "Odundun odo" [8]. It has an erect or branched stem and can be as tall as 1.40 m. The leaves are usually green with petiole within the range 1.50 – 3.00 cm, pilose with a measurement of 4.00 – 6.00 cm [9]. It is native to West Africa and was originally named in Sierra Leone and Nigeria. Subsequently, it has been widely distributed in Cote d'Ivoire, Ghana, Guinea, Liberia, and Taiwan as well as in the Caribbean [10,11,12,13]. It is disseminated by seed and grows on fallow land, swamps, farmland and roadside. Synonyms of *Emilia praetermissa* exist as *Emilia coccinea* (Sims) G. Don, *Emilia fosbergii* (Nicolson), *Emilia, mbagoi (*Beentje & Mesfin), *Emilia reddyi* (Satish & J.Prak.Rao), *Emilia sonchifolia* (Linn.) DC and *Emilia papuana (Mattf)* [10]. Classes of phytochemicals associated with *Emilia praetermissa* have been identified by chromatographic techniques [14]. Locally the leaves are consumed as vegetable in soups or added to salad. It is used to treat internal and external wounds, *Otitis media* and colic. Decoction from the leaf is used to bath new born babies to prevent infections. Pharmacologically, *Emilia praetermissa* has been shown to possess anti-ulcer and antimicrobial properties [15,16], hepato-protective effect [17,18] and reduces hyperlipidemia [19].

Phytochemicals and identified compounds from the methanol leaf extract have been reported [20,21], while the total phenolic and flavonoid contents and antioxidant potential of the extract from *E. praetermissa* have also been reported [22], but information on it fractions are lacking. Thus, this study aims to further evaluate qualitatively phytochemicals in the extract and fractions, limit the quantitative determination of the phytochemicals to total flavonoids and phenolic contents, and evaluate their 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity in different solvent system.

MATERIALS AND METHODS

Collection, identification and preparation of the plant

Fresh plants of *E. praetermissa* were collected in October, between the hours of 7:00 am-8:30 am within the confines of University of Benin, Ugbowo Campus, Benin City, Edo State. It was identified by Prof E.I. Aigbokhan in the Department of Plant Biology and Biotechnology, with herbarium voucher number UBH-E407 and specimen was deposited for future reference. The leaves were carefully removed and air dried away from direct sunlight for 25 days. It was crushed and reduced to fine powder with the aid of an electric milling machine and stored in an air tight container until further work was done.

Extraction and Fractionation

Extraction was done with the aid of a Soxhlet apparatus for 18 h, using methanol (2.5 L) and 500 g of powdered leaves. The extract was concentrated using a rotary evaporator *in-vacuum* at 50 ^{o}C and the weight of the extract was noted (30 g). The crude extract (20 g) was measured and dissolved in 100 mL of methanol and 400 mL of water. The mixture was partitioned in a separating funnel with 4x500 mL of n-hexane. The collected n-

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hexane fractions were bulked together and concentrated using a rotatory evaporator. This procedure was repeated for dichloromethane and ethylacetate respectively and their concentrated fractions were kept 4 ^{o}C until used.

Phytochemical Screening of Crude Extract and Fractions of *Emilia praetermissa*

Phytochemical screening of the crude extract and fractions was carried out in accordance with Sofowora, Harbone and Evans [23,24,25] protocols. Phytochemicals evaluated include alkaloids, saponins, tannins, phenolic compounds, terpenoids and flavonoids.

Preparation of Stock and Standard Solutions

Gallic acid and Quercetin Stock solution were prepared by solubilizing 0.02 g of gallic acid and quercetin in separate analytical flasks with capacity of 10 mL, distilled water was added to mark. This was subsequently diluted to the required concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL and 12.5 µg/mL respectively, by diluting the stock with equivalent quantity of distilled water to make up to required volume which corresponds to each concentration.

Ascorbic acid standard was used as the standard to ascertain the DPPH radical scavenging potential; the concentration of the stock solution of ascorbic acid, extract and fractions were prepared in 1 mg/mL. This was diluted to different concentration of 3.25 mg/L to 120mg/L.

Determination of Phenolic Content

The total phenolic content was determined by the method described by Kim and co-workers [26]. Concentration (1 mg/1 mL) of the extract and fractions were prepared in methanol, furthermore 0.5 mL of this solution was measured into a test tube and 4.5 mL of distilled water was added as well as 0.5 mL of Folin Ciocalteu's reagent (diluted with water 1:10 v/v). After this the test tubes were maintained at room temperature for 5 min, followed by addition of 5 mL of 7 % sodium carbonate and 2 mL of distilled water. The mixture was incubated for 90 min at room temperature after swirling. A spectrophotometer was used to measure the absorbance at 750 nm. The standard used was Gallic acid in five different concentrations of 12.5, 25, 50, 100 and 200 µg/mL in triplicate.

Evaluation of Total Flavonoid Content

The total flavonoid content was estimated using a method described by Ebrahimzadeh and co-workers [27]. A volume of 0.5 mL of the sample extract and fractions (1 mg/mL) were individually mixed with 1.5 mL of methanol, 0.1 mL of 10 % aluminum chloride, 0.1 mL of 1.0 M potassium acetate and 2.8 mL of distilled water. The mixtures were incubated for 30 min and absorbance was measured at 415 nm spectrophotometrically. The standard used was quercetin in five different concentrations of 12.5, 25, 50, 75, 100 and 200 µg/ml in triplicate.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity

The scavenging effects of extract and fractions on DPPH radical were estimated with method described by Jain and coworkers [28]. A solution of 0.1 mM DPPH in methanol was prepared, and 1.0 mL of this solution was mixed with 3.0 mL of extract in methanol containing 0.001 - 0.2 mg/mL of the extract. The reaction mixture was vortexed thoroughly and left in the dark at

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room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm.
Ascorbic acid was used as reference standard. The ability to scavenge DPPH radical was calculated by the equation below:

Percentage Radical Scavenging Activity (%) = A_o - A_1/ A_o…….. (1)

Where: A_o = Absorbance of DPPH radical + methanol; A_1 = Absorbance of DPPH radical + sample extract (fractions) / standard.
The 50 % inhibitory concentration value (IC50) was extrapolated as the concentration from the 50 % point mark on the plotting of percentage inhibition against the concentration of the extract or fractions.

Statistical Analysis

Quantification of phenols and flavonoids, and antioxidant determination experiments were done in triplicate and results expressed as mean ± SEM. The data were analyzed by one way ANOVA and level of significance was set at $P < 0.05$, Turkey posthoc analysis was done and statistical package used was version 5.01 (Graphpad Prism).

RESULTS

Phytochemical Screening

The qualitative phytochemical screening of the extract showed alkaloid, tannins, saponin, flavonoid, terpenoids and phenolic compounds as inferred in Table 1. Alkaloids and flavonoids were common occurrence in the fractions, while saponins was absent in the extract and fractions. Steroids are absent in the semi-polar fractions, terpenoids were also recorded to be absent in the ethylacetate fraction.

Table 1 Phytochemical Screening of extract and fractions of *Emilia praetermissa* leaves

Phytochemicals	Methanol extract	N-hexane fraction	Ethylacetate fraction	Dichloromethane fraction
Alkaloids	+	+	+	+
Tannins	+	-	+	+
Saponins	-	-	-	-
Flavonoids	+	+	+	+
Phenolic compound	+	+	+	+
Steroids	+	+	-	-
Glycosides	+	-	+	+
Terpenoids	+	-	-	+

Key; Present = +, Absent = -

Total phenolic and flavonoids content

The quantitative analysis of the total phenolic contents of the extract and fractions showed that dichloromethane has the highest phenolic content of (60.94 ± 0.2224) mgGAE/g, this was followed by ethylacetate (35.33 ± 0.15) mgGAE/g, methanol extract (23.24±0.07) mgGAE/g and n-hexane (20.42±0.06) mgGAE/g respectively. Total flavonoid content (TFC) was highest in dichloromethane 19.43±0.06 mgQE/g and ethylacetate fractions 13.37±0.16 mgQE/g.

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Table 2 Total phenolic and flavonoids content of extract and fractions of *Emilia praetermissa* leaf

Extract/ Fraction	Phenolic Content (mgGAE/g)	Flavonoid Content (mgQE/g)
Methanol	23.24±0.07	9.89±0.23
N-hexane	20.42±0.06	6.42±0.06
Dichloromethane	60.94±0.22	19.43±0.06
Ethylacetate	35.33±0.15	13.37±0.16

DPPH Radical Scavenging Activity

Analyzing the DPPH radical scavenging effect, for both extract and fractions indicated a gradual increase in the percentage scavenging effect for all the assays. However significant difference were observed at 3.25 µg/mL, 15 µg/mL, 60 µg/mL and 120 µg/mL, when methanol extract and n-hexane fraction were compared to ascorbic acid at $p < 0.05$; also comparing the fractions-n-hexane: dichloromethane, showed some level of significant difference at a concentration of 60 µg/mL ($p < 0.05$); At 120 µg/mL, the level of significant different for n-hexane when compared to methanol was observed to be $p < 0.05$. IC50 (potency) of the extract and fractions were studied and dichloromethane fraction gave the highest value of 30.00 µg/mL, this was closely followed by methanol extract with 58.00 µg/mL, ethylacetate fraction then followed with IC50 of 98.20 µg/mL, while n-hexane fraction was the less with IC50 of 117.00 µg/mL

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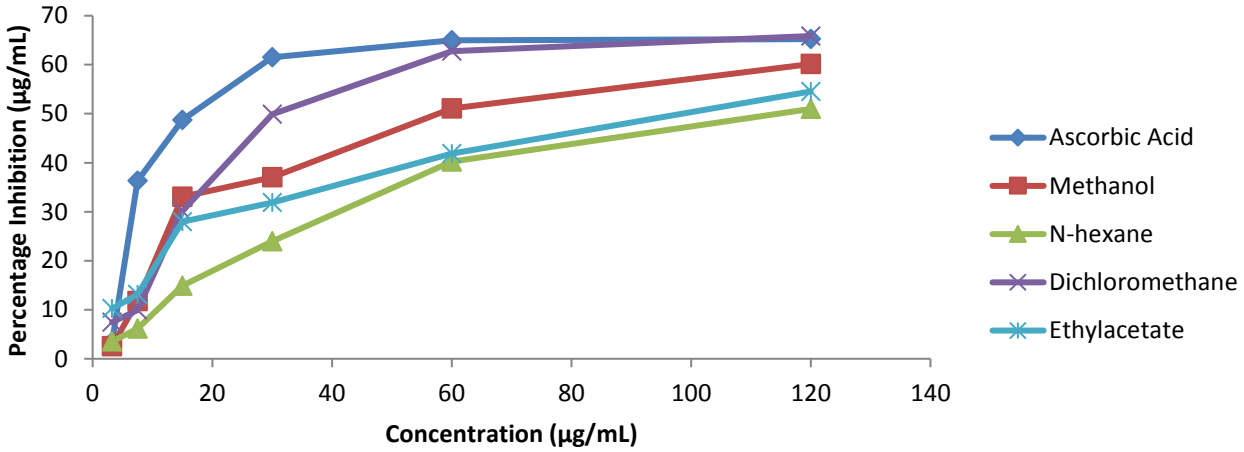

Figure 1 Percentage inhibition of DPPH scavenging activity of extract and fractions of *Emilia praetermissa* leaf

Calibration curve of gallic acid is as presented in figure 1, with R^2 of 0.9981, indicating that the plot started from the origin.

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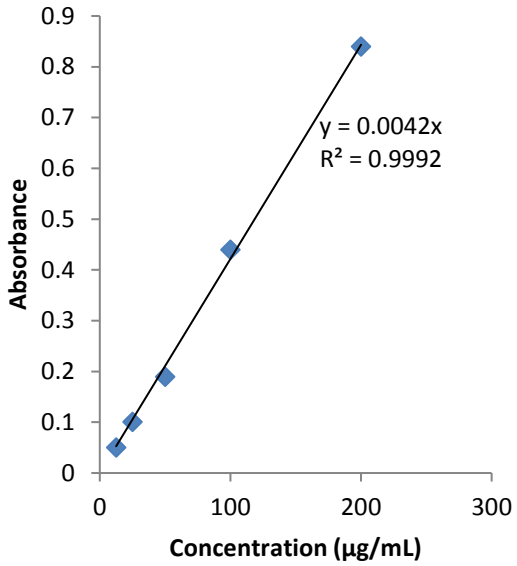

Figure 2: Gallic acid calibration curve
Quercetin calibration plot indicated R^2 of 0.9979 with graph starting from the origin

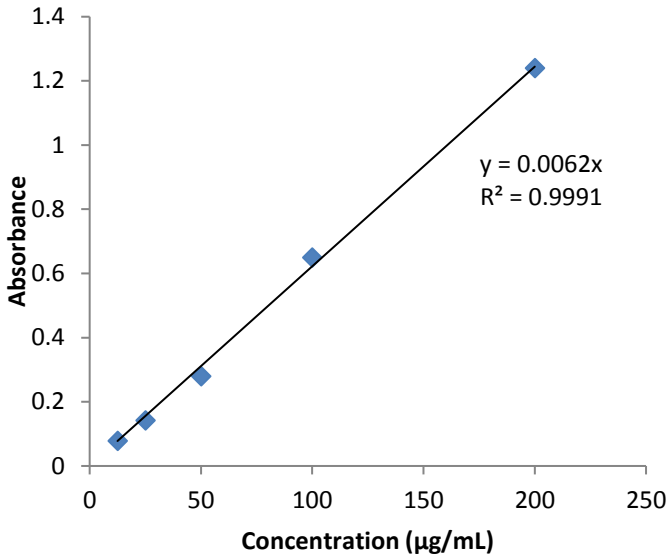

Figure 3: Gallic acid calibration curve

DISCUSSION

Phytochemical screening provides relevant understanding of the identity of bioactive constituents in a plant, leading to drug discovery, validating it traditional medicine usage and aid in the indulging of plant base health benefit [29]. Thus, knowledge of the phytochemical constituent in *Emilia praetermissa* leaf provides an insight into some reasons behind its ethno medicinal application. For instance, *E. praetermissa* leaf is use traditionally for the treatment of bacterial infection; this has been linked to polar phytochemicals [21]. Prominent polar phytochemicals include phenolic compounds and flavonoids, which contain at least one hydroxyl group attached to benzene ring. The phytochemicals identified from the methanol extract in this study have been previously screened [15], where ethanol extract was tested. In a later study, Ikezu [21] reported the presence of tannins in the n-hexane leaf fraction of *E. praetermissa*, following successive extraction while this study tested negative for tannins, this variation could be due to liquid-liquid partitioning of extract using gradient solvent. This study sequentially extracted and partitioned the extract into non-polar and semi-polar solvents, while Ikezu extracted the powdered drug with non-polar, semi-polar and polar solvents respectively, before screening for phytochemicals.

Health benefit of a plant have been tightening to phytochemicals, two phytochemicals (phenols and flavonoids) identified from the screening, could be quantified by documented spectroscopic protocol. This involves an interaction between phenol and Folin Ciocalteu reagent resulting in a reduced blue complex of phosphotungstic and phosphomolybdic acid. The intensity of this

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complex is dependent of the concentration of the phenolic contents. High level of phenols in the polar solvents; dichloromethane and ethylacetate were reported as against the non-polar solvent, considering that solvent play important role in the availability of these phytochemicals in *invitro* assay. Study conducted by Smith [30] showed the extent of phenolic compounds that were obtained from the methanol extract of *E. praetermissa*, this was far below that obtained from this study. Perusing through the study showed difference in extractive procedure and extractive solvent, Soxhlet extractive method used in this study has the advantage of exhaustive extraction compared to maceration, which may have contributed to the high value witness in this study. Similarly, pattern was observed for the flavonoid contents, relative closeness in structure between phenolic compounds and flavonoids could be responsible. Aluminum chloride forms a stable complex with carbon-4 ketone group and either carbon-3 or carbon-4 hydroxyl group in flavones and flavonols, this can be measured colorimetrically in it *in-vitro* quantification assay. Base on their structural features, flavonols and flavones are good antioxidant agents, though variations exist significantly in structure of their co-geners [31,32]. Phenols and flavonoids are important secondary metabolites, characterized by the presence of hydroxyl group in their molecule [33,34]. They have been linked to many favorable effects seen in the consumption of herbs which could be significantly due to their antioxidant ability [35], thus they are known as natural antioxidants that plays important role in the wellbeing of human. Research has indicated that phenolic compounds are more abundant in comparison to other dietary antioxidant such as alkaloids, tannins and terpenoids [36,37,38], this agrees with this present study.

Radical scavenging activity is based on the ability of the antioxidant to hunt for free radicals produced by DPPH [39]. It is a reduction reaction that results in the de-colorization of the purple color of DPPH molecule to yellow. Purple color associated with DPPH molecule is the consequence of delocalized or unpaired electron, impacting a maximum absorption wavelength of 517 nm on the molecule; also, DPPH lacks the ability to dimerize unlike many other free radicals [40]. The structure of an antioxidant molecule determines its inherent reactivity towards free radicals and other reactive oxygen species (ROS) [41]. A common standard use in antioxidant assay is ascorbic acid, it donates a proton to the free radical, which produce a stable form of DPPH measured colorimetrically. Different extracts of *E. praetermissa* have been reported with antioxidant potentials, which compared well with ascorbic acid standard which provided a basis for advance evaluation [23]. This study further evaluated the fractions of the methanol extract and showed that the fractions possess better activity as concentration increases. This could be due to partial purification of the extract, through the partitioning process. The IC_{50} showed that dichloromethane fraction is the most potent of the extract and fractions though double that of ascorbic acid. This indicate that 30.00 µg/mL is required to inhibit 50 % of the action of free radicals produce by DPPH.

CONCLUSION

This study was able to identify the different phytochemicals in the extract and various fractions, of these phytochemicals, phenols

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and flavonoids content were estimated spectroscopically and free radical scavenging ability of these phyto-constituents were determined. The dichloromethane and ethylacetate fractions showed improved antioxidant potential when compared to the methanol extract, indicating that partial purification of extract aids in improving potency as may have been observed from the IC_{50} value recorded. This further validate the ethno-medicinally use of *E. praetermissa* in the management of oxidativeassociated diseases.

Conflict of interest: Not applicable

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Authors Contributions: Beauty Ighomena collected data, analysis of data and wrote the draft of the manuscript. Emmanuel Eimiomodebheki Odion Conceptualized, supervised and proofread the manuscript

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